

## **SOLID-LIQUID PARTITIONING OF SARS-COV-2 AND ENTERIC VIRUSES PRESENT IN SEWAGE IN HIGH-RATE ALGAL POND AND USE OF THERMAL DISINFECTION IN ALGAL BIOMASS**

Rosa-Machado, A.\*, Vassalle, L.\*, Lopes, B.\*, Espinosa, M\* and Mota, C

\* Federal University of Minas Gerais. e-mail: cesar@desa.ufmg.br

Highlights:

- The system UASB reactor+HRAP is efficient for removal of viruses
- Solar collector systems are efficient for disinfecting algal biomass;
- The SARS-CoV-2 of left the system in the UASB sludge only;
- More than 50% RV-A and HAdV of left the system in the solid phase.

Keywords: SARS-CoV-2; Rotavirus A; human adenovirus.

### **INTRODUCTION**

The presence of enteric viruses in domestic sewage is a major concern due to their diversity and high transmission capacity. In a global context, there is also concern about the presence of the SARS-CoV-2 virus in sewage, although the fecal-oral route for Covid-19 transmission has not yet been proven. In Brazil, upflow anaerobic sludge blanket (UASB) reactors are one of the most widely used technologies for sewage treatment (ANA, 2020). However, the final effluent from these reactors often does not meet the standards imposed by legislation, requiring post-treatment.

High-rate algal ponds (HRAP) allow for aligning the post-treatment of anaerobic effluents with the production of nutrient-rich biomass, which can be used as a component for producing feed and biofertilizers (Passos et al., 2018). Furthermore, these systems can achieve high removal of pathogens and micropollutants present in sewage (Espinosa et al., 2022; Vassalle et al., 2020). However, part of the pathogens present in the effluent may be adsorbed with the biomass (Espinosa et al., 2022). To safely reuse this microalgae, one alternative is to apply heat treatment using solar collectors.

However, there are gaps in the literature regarding the removal of viral pathogens and SARS-CoV-2 in UASB + HRAP systems, as well as the interaction of these pathogens with the algal biomass produced during treatment. In this context, the present work evaluated the removal, reduction, and solid-liquid partitioning of SARS-CoV-2, Rotavirus A (RV-A), and Human Adenovirus (HAdV) in a treatment system composed

of a UASB reactor followed by two HRAPs operating in parallel, and the use of solar collectors for thermal disinfection of biomass.

## METHODOLOGY

**Experimental design and samplings:** The UASB reactor used in the study has a working volume of 343 L and was operated at a flow rate of 50 L.h<sup>-1</sup>, resulting in a hydraulic retention time (HRT) of 7 hours. The two HRAPs operated in parallel, each with a volume of 205 L, at a flow rate of 25.5 L.day<sup>-1</sup> and an HRT of 8 days. The biomass settler has a volume of 30 L and an HRT of 14 hours. The settled biomass was sent to a solar thermal treatment unit and subsequently reintroduced into the UASB reactor to be co-digested together with the raw sewage. The solar treatment unit was composed of four commercially available vacuum tempered glass tubes, each with a working volume of 3 L, and a fiberglass-insulated reservoir with a useful capacity of 16 L. The total capacity of the system was 28 L. The treatment unit was operated with an HRT of 13 hours. The sampling and monitoring period took place between September and December 2020, during the COVID-19 pandemic, totaling 10 samplings for both the liquid and solid phases. The experimental unit and sampling points are shown in Figure 1.

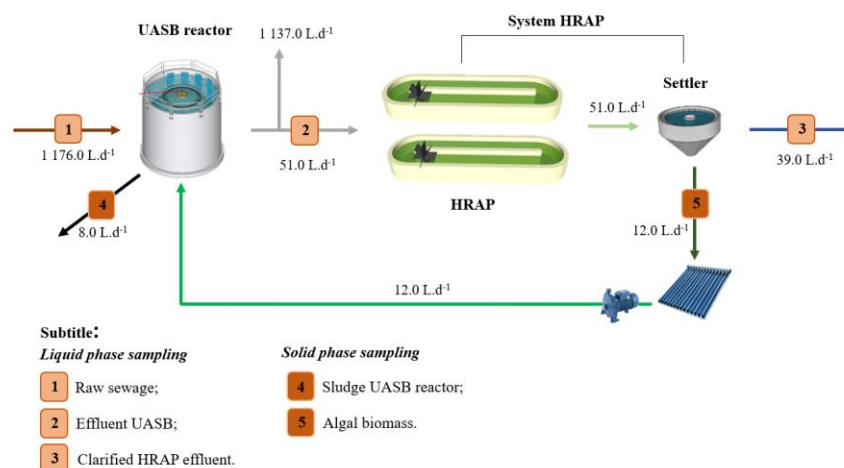


Figure 1: Experimental unit (UASB reactor + HRAP) and liquid and solid phase sampling points.

**Molecular quantification:** The viral DNA/RNA concentration method was performed as described by Ahmed et al. (2020b), which is a modification of the adsorption-elution method described by Katayama et al. (2002). The analyses for detection and quantification of SARS-CoV-2, Rotavirus A (RV-A), and Human Adenovirus were carried out using the quantitative polymerase chain reaction (qPCR) technique.

For the quantification of SARS-CoV-2 and Human Adenovirus, the assay described by Espinosa et al. (2022) was used. For RV-A, a SYBR-green assay was employed, using primers recommended by Applied Biosystems™ (Forward: ACCCTCTATGAGCACAATA, Reverse: GGTCACATAACGCCCTA). For reverse transcription, the MasterMix Itaq Universal Probes One Step kit (Biorad) was used.

Analysis of removal, reduction, and partitioning of the virus: The removal, reduction, and liquid-solid partitioning of the viruses were calculated according to the model presented by Espinosa et al. (2021).

## RESULTS AND CONCLUSIONS

The average concentrations of SARS-CoV-2, RV-A, and HAdV in raw sewage were 3.9, 3.72, and 4.73 log copies.L<sup>-1</sup>. No significant removal of the viruses was observed in the UASB reactor. However, after the post-treatment of anaerobic effluent by the HRAPs, a possible total removal (absence of the virus in the effluent) of SARS-CoV-2 was observed, estimated at 3.9 logs. For RV-A and HAdV, the removals were 0.29 and 2.72 logs, respectively. The average global reduction in the UASB+HRAP system was 1.27 logs for SARS-CoV-2 ( $p < 0.05$ ). For RV-A and HAdV, the system did not show statistically significant reductions. Finally, it was observed that most of the viruses left the system adsorbed to the UASB sludge and algal biomass.

After applying thermal disinfection using solar collectors for 13 hours, a possible total removal of RV-A and HAdV adsorbed to the microalgae was observed, estimated at 3.98 and 2.68 log copies.L<sup>-1</sup>. The average temperature in the system was 37°C.

These results demonstrate the potential effectiveness of HRAPs in the removal of SARS-CoV-2 and highlight the importance of solar thermal treatment for the elimination of other remaining enteric viruses in the biomass, reinforcing its potential viability as a safe alternative for the reuse of treated effluents and algal biomass. However, the limited removal of RV-A and HAdV in the UASB+HRAP system underscores the need for process improvements, especially to increase efficiency in the removal of these viruses under real operational conditions. Finally, further studies are needed to assess the viability of viruses that leave the UASB reactors adsorbed to the sludge. Moreover, it is essential to investigate and develop effective treatment methods for this sludge, in order to ensure pathogen elimination and guarantee that its use is safe and sustainable.

## ACKNOWLEDGMENTS

This study was supported by the National Science Foundation (USA) under Grant No. 1827251 and the following Brazilian organizations: National Research Council – CNPq; to the National Institute of Science and Technology on Sustainable Sewage Treatment Plants - INCT ETEs Sustentáveis; Coordination for the Improvement of Higher Education Personnel – CAPES; Foundation for Research of the State of Minas Gerais – FAPEMIG (Brazil); the National Health Foundation – FUNASA (Brazil), Rosa-Machado is oriented by C.R. Mota Filho.

## REFERENCES

ANA–National Water Agency, 2017. Watershed Sewage Depollution Atlas. Available at: <http://atlasesgotos.ana.gov.br>.

Ahmed, W. et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. *Science of the Total Environment*, v. 728, p. 138764, 2020b.

Espinosa, M. F. et al. Reduction and liquid-solid partitioning of SARS-CoV-2 and adenovirus throughout the different stages of a pilot-scale wastewater treatment plant. *Water research*, p. 118069, 2022.

Katayama, H., Shimasaki, A., Ohgaki, S., 2002. Development of a virus concentration method and its application to detection of enterovirus and Norwalk virus from coastal seawater. *Appl. Environ. Microbiol.* 68, 1033–1039. <https://doi.org/10.1128/AEM.68.3.1033-1039.2002>.

Passos, F. et al. Anaerobic co-digestion of coffee husks and microalgal biomass after thermal hydrolysis. *Bioresource Technology*, v. 253, n. December 2017, p. 49–54, 2018.

Vassalle, L. et al. Can high rate algal ponds be used as post-treatment of UASB reactors to remove micropollutants? *Chemosphere*, v. 248, 2020b.